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Reverse transcriptase-polymerase chain reaction for expression of tyrosinase to identify malignant melanoma cells in peripheral blood.

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BACKGROUND: Circulating tumour cells in the peripheral blood may be important for haematogenous spread of disease. The detection of these cells may therefore be a poor prognostic indicator. Reverse-transcriptase polymerase chain reaction (RT-PCR) of target tumour-specific protein expression has been used as a sensitive and specific method for the detection of these tumour cells. Initial reports by our laboratory and other suggested RT-PCR amplification of the enzyme tyrosinase is a useful method for detection of melanoma cells in peripheral blood [1-3]. PATIENTS AND METHODS: In this report, we have evaluated the application of RT-PCR for tyrosinase mRNA as a detection method for melanoma cells in a series of 24 patients with advanced, metastatic malignant melanoma. A single round RT-PCR method is described. RESULTS: The single round RT-PCR was as sensitive as previously described nested PCR methods, and had the advantage of reduced contamination risks. Blood samples from three out of the twenty-four patients were positive. CONCLUSIONS: The frequency of tumour cell detection in peripheral blood from patients with advanced disease was lower than previously reported. It may be only small numbers of circulating tumour cells are present at any one time in the peripheral blood of patients with malignant melanoma. If this is the case increased sampling will improve detection frequency. Alternatively, dissemination of melanoma through peripheral blood may be a rare event. In our experience, RT-PCR for tyrosinase mRNA as a staging test for melanoma patients must be interpreted cautiously.

PMID: 8740795 [PubMed - indexed for MEDLINE]

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Use of a reverse-transcriptase polymerase chain reaction for monitoring the shedding of feline coronavirus by healthy cats.

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The pattern of shedding of feline coronavirus (FCoV) was established in 155 naturally infected pet cats from 29 households over periods of up to five years. Viral RNA was detected in faeces by reverse-transcriptase PCR (RT-PCR), and plasma antiviral antibodies by immunofluorescence. The cats rarely shed FCoV in their saliva. Three patterns of FCoV shedding were observed. Eighteen of the cats shed virus continuously, so were persistent, and possibly lifelong, carriers; none of them developed feline infectious peritonitis. Fifty-six cats ceased shedding virus, although they were susceptible to reinfection, and 44 shed intermittently or were being continuously reinfected. Four of the cats were resistant to infection. Seventy-three per cent of the virus shedding episodes lasted up to three months and 95 per cent up to nine months. There was a correlation between shedding and antibody titre but the cats could remain seropositive for some time after they had ceased shedding virus. One-off testing for FCoV by RT-PCR is inappropriate. Identification of longterm carriers requires that a positive result be obtained by RT-PCR on faecal samples for at least eight consecutive months. A cat should be shown to be negative over five months, or to have become seronegative, to ensure that it has ceased shedding virus.

PMID: 11400984 [PubMed - indexed for MEDLINE]

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Reproducibility of detection of tyrosinase and MART-1 transcripts in the peripheral blood of melanoma patients: a quality control study using real-time quantitative RT-PCR.

de Vries TJ, Fourkour A, Punt CJ, van de Locht LT, Wobbes T, van den Bosch S, de Rooij MJ, Mensink EJ, Ruiter DJ, van Muijen GN.

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In recent years, large discrepancies were described in the success rate of the tyrosinase reverse transcription polymerase chain reaction (RT-PCR) for detecting melanoma cells in the peripheral blood of melanoma patients. We present a quality control study in which we analysed the reproducibility of detection of tyrosinase and MART-1 transcripts in 106 blood samples from 68 melanoma patients (mainly stages III and IV). With this study, we aimed to improve insight in the reproducibility of a RT-PCR for the detection of (minimal) amounts of circulating melanoma cells. We performed two reverse transcriptions on each mRNA sample and performed tyrosinase and MART-1 nested PCRs in duplicate per cDNA sample. Thus, four tyrosinase and four MART-1 measurements were performed per blood sample. In our study, the majority of blood samples was negative for tyrosinase (80%) or MART-1 (66%). Only four samples were positive in all four determinations for tyrosinase and seven for MART-1. Variable results (1-3 times positive results) were obtained for tyrosinase and MART-1 in 16% and 27% respectively. MART-1 PCR had a better performance than tyrosinase PCR. Sensitivity increased when both markers were used. We reasoned that the low number of melanoma marker PCR-positive blood samples can be explained by differences in mRNA quality. By using real-time quantitative PCR, we found that this was not the case: amplification of porphobilinogen deaminase (PBGD), a low copy household gene, was not different in blood samples in which a melanoma marker was not detected from groups in which this marker was detected more or less consistently (1-4 times). When applying real-time quantitative PCR for tyrosinase and MART-1, we found that a low amount of SK-MEL-28 cell equivalents was present in the blood of melanoma patients, with a higher number of equivalents in the group with a consistently positive result. We conclude that low reproducibility of a repeated assay for the detection of circulating melanoma cells is not caused by differences in mRNA quality between the samples, but due to low numbers of amplifiable target mRNA molecules in the

mRNA sample. Use of more than one marker and repetition of the assay will increase the probability of finding positive PCR results.

PMID: 10360670 [PubMed - indexed for MEDLINE]
